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Detection and subtype identification of Blastocystis in a hospital setting from southeasternIndia

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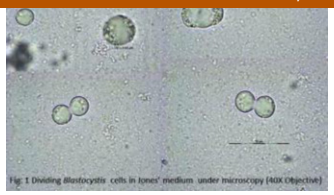
Background: *Blastocystis* was identified almost 100 years back but its clinical significance is controversial. An estimate suggests that *Blastocystis* inhabit large intestine of more than 1 billion human worldwide. Based on the ribosomal lineages different species of *Blastocystis* are designated as various subtypes (ST) with extensive inter and intra subtype genetic diversity. Due to its polymorphic nature identification by microscopy is obscure. However, in India, data pertaining to *Blastocystis* were chiefly derived from direct stool microscopy. In this study we employed microscopy, culture and PCR for the detection of *Blastocystis* from stool samples. Further, subtyping of representative samples were carried out to identify the subtypes available in this region.

Methods & Materials: It is a cross sectional analytical study approved by JIPMER Institute Ethics Committee. All the stool samples were screened by routine microscopic investigations and they were subjected to *in vitro* propagation in Jones' medium. Fecal DNA was extracted by using QIAamp DNA stool mini kit (Qiagen, Germany) following manufacturer's instructions and stored at -20°C. Further, extracted DNA was quantified and subjected to PCR, which targets initial 600 bp barcoding region of 18SSU rDNA of *Blastocystis*. PCR products were visualized on 1.5% agarose gel and representative positive amplicons were sequenced for subtype analysis. Sequence results obtained from both the strands were assembled and subtype analysis was performed by using following database http://www.pubmlst.org/blastocystis/

Results: A total of 173 stool samples were screened for *Blastocystis*. PCR detected the maximum number of *Blastocystis* (n = 77, 44%) followed by culture (n = 48, 28%) and Microscopy (n = 25, 14%). The Sequencing results of the representative PCR amplicons confirmed the presence of *Blastocystis* ST3 allele 34 (n = 9) and ST1 allele 4 in (n = 5).



Conclusion: In comparison with stool microscopy and culture, *Blastocystis* specific PCR is an excellent diagnostic tool in terms of sensitivity, specificity and subtype identification. However, in resource poor settings Jones' medium (xenic culture) could be used as an alternative diagnostic modality for the detection of *Blastocystis* from stool. Subtyping results indicate ST3 predominance. However, large number of samples needs to be subtyped to reveal the association of particular ST with particular clinical manifestations.

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MicroRNA mediated immune regulation of T helper cell differentiation and plasticity during visceral leishmaniasis infection: A computational approach

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Background: Visceral leishmaniasis (VL) is a tropical neglected disease caused by *Leishmania donovani*, results in significant mortality in Indian subcontinent. The protective immune response to *Leishmania* parasites is mediated by proliferation and differentiation of IFN-γ secreting $CD4^+$ T helper (Th1) cells while IL-4 dependent $CD4^+$ T helper (Th2) cell leads to aggravate VL pathogenesis. The plasticity of T cell proliferation and differentiation depends on microRNA mediated gene regulation which leads Th1/Th2 or Th17/Treg type of immune response during human VL.

Methods & Materials: MicroRNAs participates in T cell proliferation and differentiation in human VL. This study depicts the identification of target immune signaling molecule and transcription factors, which play role in T-cell proliferation and differentiation followed by the identification of miRNA controlling their gene expression using three web servers viz., TargetScan, mirPath and miRDB.

Results: The present study provides the *in silico* evidences that seed region present in the microRNAs miR-29-a, miR-29b and miR29c have the putative binding site in the 3'-UTR region of TBX21 transcription factor of $CD4^+$ T helper (Th1), which may suppress the Th1 specific protective immune response. Development of Th2 type specific immune response can be suppressed by binding of miR-135 microRNA over the 3'-UTR region of GATA-3 transcription factor of Th2 specific $CD4^+$ T helper cells. Interestingly, miR-21 and miR-24